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Photosensitisers – the progression from photodynamic therapy to anti-infective surfaces

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Abstract

Introduction: The application of light as a stimulus in pharmaceutical systems and the associated ability to provide precise spatiotemporal control over location, wavelength and intensity, allowing ease of external control independent of environmental conditionals, has led to its increased use. Of particular note is the use of light with photosensitisers.

Areas covered: Photosensitisers are widely used in photodynamic therapy to cause a cidal effect towards cells on irradiation due to the generation of reactive oxygen species. These cidal effects have also been employed to treat infectious diseases. The effects and benefits of photosensitisers in the treatment of such conditions are still being developed and further realised, with the design of novel delivery strategies. This review provides an overview of the realisation of the pharmaceutically relevant uses of photosensitisers both in the context of current research, and in terms of current clinical application, and looks to the future direction.

Expert opinion: Substantial advances have been and are being made in the use of photosensitisers. Of particular note are their antimicrobial applications, due to absence of resistance that is so frequently associated with conventional treatments. Their potency of action, and the ability to immobilise to polymeric supports is opening a wide range of possibilities with great potential for use in healthcare infection prevention strategies.

1. Introduction

Light and its benefits have long been studied by many different disciplines, and its use has been extended to the initiation and facilitation of reactions with benefit to health when applied to pharmaceutical and antimicrobial applications. This review provides an overview of the use of light in conjunction with photosensitisers, and the progress that has been made from initial experimentation to clinical application and commercialisation of photosensitising systems.

2. Photodynamic therapy

It has long been known that light can be used in conjunction with photoreactive plant-based compounds for medicinal benefit, most notably for skin hyperpigmentation. Ancient Egyptians, from as early as 2000 B.C., used the juice of furocoumarin-containing *Ammi majus* to topically treat patches of vitiligo, followed by sun exposure, and the ancient Indian Ayurvedic system of medicine describes the use of seeds from the psoralen-containing *Psoralea corylifolia* to treat leucoderma

[1]. Phototherapy, whereby light is applied to the body to cause a change, often due to absorption by photosensitive compounds within the body, was then, and still is, used as a medical treatment for a number of conditions. In the 1950s, neonatal jaundice was treated with the application of blue light, with the probable mechanism of action being realised substantially more recently as the *cis-trans* isomerisation around one of the bonds in bilirubin [2]. Its use in acne is attributed to the activation of a photosensitiser, coproporphyrin III in the bacterium *Propionibacterium acnes* [3, 4]. Whilst phototherapy finds utility in some cases, it requires that the area to be treated is itself responsive to visible light, therefore limiting its use. It is possible, however, to employ the use of exogenous photosensitive compounds that can be applied internally or externally, to allow treatment of disease. This has been termed photodynamic therapy, or PDT.

2.1 Mechanism of action of photosensitisers

Oscar Raab, working alongside Herman von Tappenier, discovered the cidal effects of acridine dye on protozoal cells in 1900 [5, 6], and three years later von Tappenier published the effects of topically applied eosin and light on skin cancer [7]. The photodynamic effect was due to dye-photosensitised photooxygenation of cellular components and peroxide accumulation [8]. The term ‘photodynamic therapy’ arose from *photodynamische Wirkung* (photodynamic effect), coined by von Tappeiner in 1907 to describe the damage of living tissue by a combination of photosensitiser, visible light and oxygen [9]. The mechanism by which photosensitive compounds and dyes caused cell death was not discovered until over two decades later due to studies on the nature of oxygen and the processes occurring on photoactivation of

dyes. Mulliken discovered that oxygen in the ground state occurred as a triplet [10, 11], then in 1931, Kautsky proposed that singlet oxygen ($^1\text{O}_2$) formed due to energy transfer from the excited photosensitiser to oxygen [12], which allowed initiation of photodynamic reactions. Further studies by a number of researchers developed the understanding of the processes involved and the currently held theory of the mechanism of action of photosensitisers [13-18], detailed in Figure 1. The main photophysical and photochemical processes involved are thought to be similar for all photosensitisers.

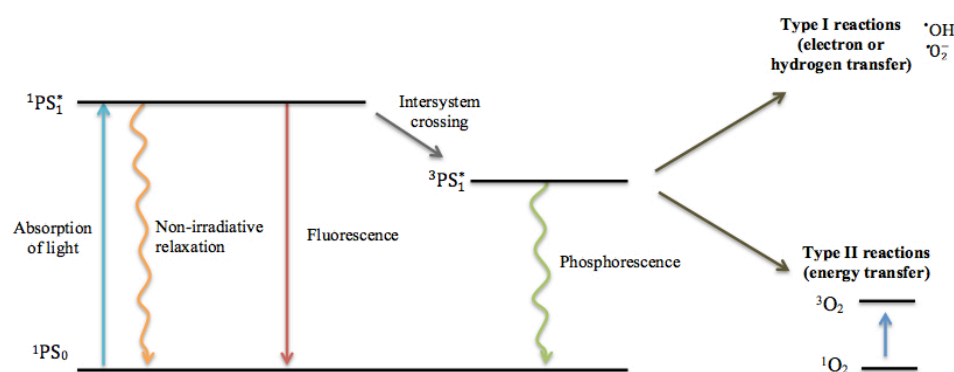


Figure 1. Jablonski diagram of the pathways leading to photosensitisation following application of light to a photosensitiser, adapted from [19]. When a photosensitiser absorbs light, it may undergo an electronic transition from the ground state ($^1\text{PS}_0$) to the singlet excited state ($^1\text{PS}_1^*$), which is short-lived. Some of the energy is then transferred, via intersystem crossing, to the relatively long-lived triplet excited state ($^3\text{PS}_1^*$). The molecule may then undergo electronic decay to the ground state, or either charge (type I reaction), or energy (type II reaction) may be transferred to a substrate or to molecular oxygen (a triplet state molecule, $^3\text{O}_2$) respectively to generate reactive oxygen species. The latter reaction is predominant. Adapted from [98].

Type I reactions lead to the generation of reactive oxygen species (ROS) due to interaction of the excited photosensitiser with a molecule in its immediate vicinity via hydrogen abstraction or electron transfer. The ROS generated may be radical cations and anions, with superoxide anions (O_2^-) being formed from electron transfer to molecular oxygen. The anions themselves are not particularly toxic to biological systems but can lead to the production of hydrogen peroxide via dismutation. Via reactions initiated by O_2^- , hydroxyl radicals can then be formed, which interact with O_2^- to generate 1O_2 .

Type II reactions involve energy transfer between an excited photosensitiser and molecular oxygen. In the ground state, due to its two unpaired electrons with parallel spins, oxygen exists as a triplet (3O_2) with a non-zero magnetic dipole. The lowest lying excited states are singlet and it is this state that is easily formed in dye-sensitised reactions (1O_2). Its lifetime is long in comparison to other ROS, estimated to be between $10^{-5} - 10^{-6}$ s [20]. 1O_2 is considered to have a predominant role in photodynamic cellular damage [21-23], mediating cytotoxic damage via damage to lysosomes, mitochondria, plasma membranes and golgi apparatuses [24].

2.2. Treatment of human conditions with PDT

Knowledge of the photophysics and photochemistry of photosensitisers has allowed the rational use of plant-based compounds, in addition to the synthetic design of photosensitisers, alongside application of light, in the treatment of human conditions. Usually the photosensitiser is either applied topically in the case of skin lesions, or is

injected and time is allowed for localisation in the site to be treated. Visible light, at a wavelength appropriate for optimum photoactivation of the photosensitiser, is then applied. For applications where treatment within deeper tissues is required, as is the case in many cancerous tumours, the photosensitiser often must absorb at a long wavelength, allowing the light applied to penetrate the body tissues. Use of fibre optics and lasers, however, has allowed treatment of tumours deeper within the body. The advantage of PDT over a number of conventional treatments, in particular over conventional chemotherapeutic treatments employed in cancer therapy, is the ability to selectively localise and accumulate the photosensitiser in the tumour or damaged tissue to be treated, and to deliver light specifically to the relevant area, thus minimising collateral damage to normal tissue. The precise mechanism of localisation is not known, but in tumours is thought to be related to the high vascular permeability of photosensitisers and their affinity for proliferating endothelial tissue, in addition to the reduced lymphatic drainage from tumours [25], and in some cases to photosensitiser binding to low-density lipoprotein (LDL) and the resultant uptake facilitated by tumour upregulation of LDL receptors [26]. As a result, particularly in cancer treatment, substantially reduced side effects are noted when compared with conventional chemotherapeutic drugs. PDT, of course, is not without disadvantage, with prolonged skin photosensitivity and excessive damage at the treatment site being reported [24], but these effects are minor when compared to those resulting from the comparatively indiscriminate ablation of cells within the body during chemotherapy.

PDT has shown success in various types of cancer, blindness due to age-related macular degeneration, and in skin actinic keratosis [27]. Whilst a large number of

photosensitisers have been researched and synthesised, a comparatively smaller number have been used clinically in disease treatment. The structures of a number of photosensitisers currently used clinically in PDT are shown in Figure 2, with the majority belonging to the porphyrin group of photosensitisers. These include haematoporphyrin derivative (HpD), the most widely used marketed photosensitiser, which exists as a mixture of oligomers. It is FDA approved for early and late endobronchial lesions, Barrett's oesophagus, and oesophageal obstructing lesions. It has also been used off-label for a variety of conditions, and has approval in many countries for the treatment of bladder, genitourinary and digestive tract cancers, as reviewed by Dougherty *et al.* [28]. High or complete responses in the FDA approved treatments have been reported [29-33], as has control of squamous and basal cell lesions and Kaposi sarcoma [34-36]. The great disadvantages of HpD are its lack of selectivity and prolonged photosensitivity, requiring patients to avoid sunlight for at least four weeks. Although it concentrates mainly in the tissue to be treated, there is also an extensive normal tissue reaction, which can manifest as swelling of the skin or necrotic tissue slough, which can be life threatening [37]. Another widely used entity for PDT is 5-aminolevulinic acid (ALA). ALA is not itself a photosensitiser, but a prodrug which is intracellularly metabolised to the photosensitiser protoporphyrin IX [38]. It, and the methylated ALA, M-ALA, have found use mainly in the treatment of skin lesions, with success reported against basal and squamous cell carcinoma, as reviewed by Klein *et al.* [39]. An ALA-containing plaster has recently been licenced by the MHRA for treatment of actinic keratosis on the face and head [40]. For a review of other photosensitisers currently marketed and used clinically, including temoporfin which poses as a potential replacement to

HpD in Europe, and some used in veterinary, reference can be made to Allison *et al.* [37].

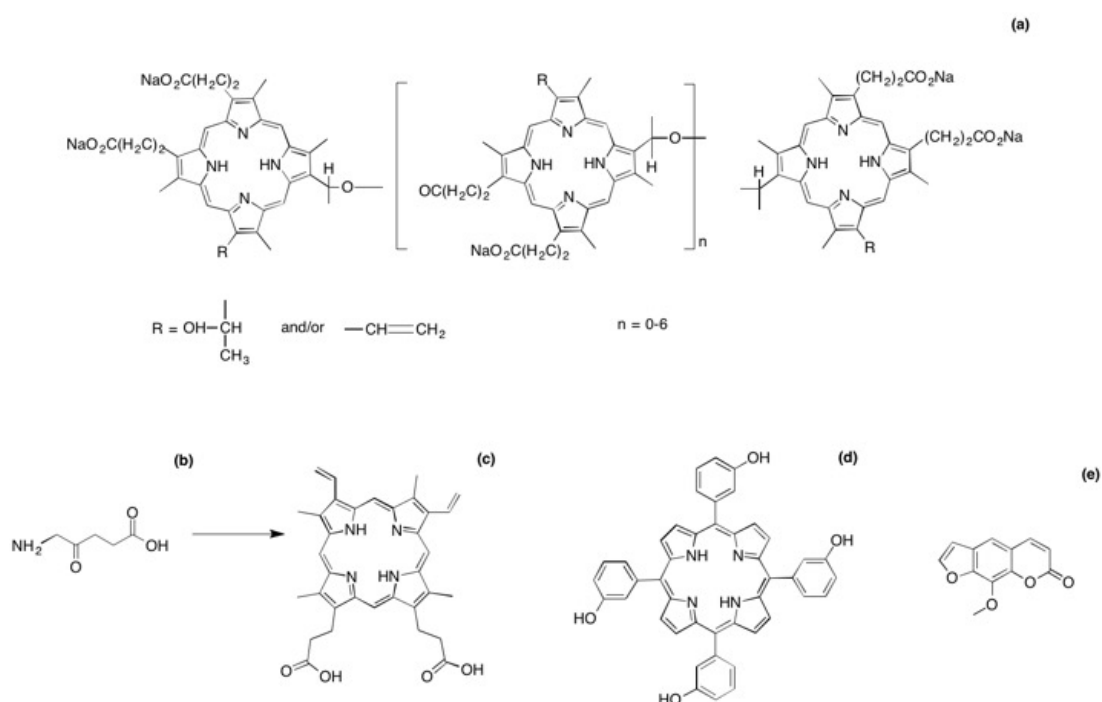


Figure 2. Examples of some photosensitisers clinically used in PDT. Haematoporphyrin derivative (a), ALA (b) and the photosensitiser to which it is intracellularly metabolised protoporphyrin IX (c), temoporfin (d), and 8-methoxypsoralen (e).

Non-porphyrin based photosensitisers are used, but to a lesser degree. One example is the continued use of psoralens in the treatment of skin lesions. Psoralen plus UVA light (320-400 nm), known as PUVA therapy was introduced in 1974 by Parrish *et*

al. [41], and is used for treatment of vitiligo, and also of psoriasis. In the latter case, light is applied following oral administration of the psoralen, 8-methoxypsoralen, with the generated reactive oxygen species causing crosslinking of DNA, therefore preventing cell replication and plaque formation. It must be noted that this does not treat the cause of the condition and therefore is not a curative intervention. PUVA therapy appears to have declined in popularity as a psoriasis treatment in recent years, perhaps due to the increasing use of narrow-band UVB phototherapy (311-313 nm). Narrow-band UVB therapy does not require the administration of a photosensitiser or wearing of eye protection, and the nausea associated with psoralens is avoided; for this reason it is often preferred by patients. Additionally, despite the comparatively more reliable and efficient clearing of psoriasis with PUVA, clinicians appear to tend towards UVB therapy due to concerns regarding the long term safety of PUVA following reports of cutaneous carcinoma [42].

The pharmaceutical application of photosensitisers, has not been limited to the treatment of human disease. Antimicrobial therapy plays a large role in the area of medicine and pharmaceuticals, and the use of light within this area has been found to be of great benefit.

3. Light-triggered antimicrobial systems

3.1 Effects of light on microorganisms

The discovery of the antibiotic, penicillin, in 1928 was one of the greatest breakthroughs in modern medicine, particularly in a culture where infection was

highly prevalent and often fatal. Prior to this, however, came another accidental discovery, also with great and long-reaching repercussions. In 1877, Downes and Blunt noted the appearance of cloudiness in sugar water placed on a windowsill in the shade but remained clear while in the sun, which microscopic examination showed to be due to bacterial growth in the solution in the shade [43]. Further studies on the effects of light on microorganisms were conducted in 1885 by Arloing [44-46] and Duclaux, who demonstrated the cidal effect of sunlight on *Bacillus anthracis* and *Tyrophthrix scaber* respectively [47]. In 1892, Geisler used a prism and heliostat to demonstrate the lethality of sunlight and electric arc lamps to *B. typhosus* [48], and in doing so provided evidence that wavelengths of all parts of the spectrum, with the exclusion of red, could cause harm to bacteria. In the same year, Marshall Ward demonstrated the UV/violet/blue portion of the spectrum primarily to have bactericidal action, conducting experiments with *Bacillus anthracis* [49]. At the end of the 19th century, Niels Finsen pioneered the use of UV light to cure lupis vulgaris in tuberculosis sufferers, and in doing so made aware the potential medical uses of light [2]. A recent study, however, has suggested that the effective wavelength possible with his lenses may have been above 340 nm, thus activating endogenous porphyrins in *Mycobacterium tuberculosis* to effect treatment, rather than UV-mediated bactericidal action [50]. Regardless of the mechanism, his invention was of great benefit, and certainly instrumental in the investigations of microbicidal effects of UV light. The precise wavelength with the most potent bactericidal effect within the UV range was further investigated by Barnard and Morgan [51] and Newcomer [52], and eventually narrowed to 253.7 nm by Ehrismann and Noethling in 1932 [47].

The discovery of the germicidal effects of UV light led to its use in drinking water disinfection [47], and in disinfection of contact lenses [53], but more significantly for the present area is its use for sterilisation within healthcare environments. As UV light cannot penetrate solid, light-absorbing materials, UV sterilisation within the hospital is mainly limited to inactivation of airborne or surface located microorganisms [54].

For a number of microorganisms, including bacterial spores, UV light alone is often not sufficient to induce a cidal effect. There are also dangers of skin and eye damage related to occupational exposure [55]. Constraining factors in the use of UV light in treatment of host-localised infections are the potential for adverse effects or lack of skin penetration. The use of visible light, however, is of greater interest, and its use for broad-spectrum antimicrobial purposes is possible due to the availability and development of photosensitisers.

3.2. Photodynamic antimicrobial chemotherapy

As discussed, the discovery of the cidal action of photosensitisers was first made in the field of microbiology by Raab [5]. The development of antibiotics in the 1940s however, stalled further research into the antimicrobial uses of photosensitisers, as bacteria could be effectively eradicated systemically and topically, with further development of antifungal, antiprotozoal and antimalarial agents. When photosensitisers were rediscovered as antimicrobials, they were first applied to lesions caused by the herpes simplex virus, but safety concerns and claims of ineffectiveness halted the progress once again [56-59]. Increasing antibiotic

resistance, however, has driven the need to find alternative antimicrobials, and photosensitisers have become a more popular topic of research.

Photodynamic antimicrobial chemotherapy (PACT) is based on PDT but applied specifically to microbial cells. The underlying principle of PACT is that if live microbial cells can be selectively demonstrated by a particular dye, which is also photosensitive, then illumination of the stained microorganism should result in a lethal action towards that cell when in a biological environment or human subject [22]. Uptake of the photosensitiser by the challenge organism is usually required, occurring in a non-specific manner (i.e. not mediated by a photosensitiser-specific uptake mechanism), followed by application of light of the appropriate wavelength. While the uptake mechanism is not specific, due to the comparatively rapid uptake of photosensitisers by microbial cells, it has been found that a short drug-light interval is optimal for selective uptake by microbial cells over the mammalian host cells and tissue [60].

Reactive oxygen species are then generated within the bacterial cell on irradiation, with the photophysics proceeding in a similar manner to that presented in Figure 1. Due to the lipophilic nature of most photosensitisers used, they can localise in the lipid membranes of the bacterial cells. The concentration of molecular oxygen in the lipid membranes is higher than in the surrounding aqueous phase, therefore favouring a type II mechanism of photodamage, and thus generation of $^1\text{O}_2$ [61, 62]. $^1\text{O}_2$ is highly reactive and can initiate further oxidative reactions with many electrophilic materials including the unsaturated lipids of the bacterial cell membrane. Oxidation of nucleic acid residues, mainly guanosine, leads to nucleotide

degradation, DNA strand breakage, and inhibition of replication [22]. The reactivity of singlet oxygen with organic molecules, however, is indiscriminate, therefore any macromolecule of the bacterial cell is a potential target. This potential multiplicity of, and lack of specificity towards, targets makes the development of resistance by bacteria more difficult [63], and indeed despite a number of attempts to induce bacterial resistance with sub-lethal PACT, no resistant organisms have yet been reported [64-67], although upregulation of a heat-shock protein has been noted in some organisms [69, 70]. This is of particular significance in light of a recent World Health Organisation (WHO) report highlighting the global problem of antibiotic resistance in bacteria [68]. A proportion of the cidal actions may also be related to the specific photosensitiser used, as this can determine the predominant photodynamic reaction (type I or II) occurring, and factors such as charge, lipophilicity, size and shape will influence their main site of interaction with and localisation within the microbial cell. Cells in the logarithmic growth phase are more susceptible than those in the stationary phase [22]. Additional factors affecting cidal activity include the wavelength and intensity of light absorption, and the efficiency of production of $^1\text{O}_2$ [71].

A wide range of bacterial species have demonstrated sensitivity to photosensitiser-mediated cidal treatment, including a number of antibiotic-resistant bacteria [72-75]. Photosensitisers have been shown to be effective antifungal [76-78], antiviral [79], and sporicidal [80-83] agents, in addition to showing promise in the treatment of tropical diseases such as malaria [84-86] and leishmaniasis [87-90].

Although PACT is effective against a number of bacterial genera and species, there are differences in susceptibility due to differences in bacterial cell wall structure. Gram-negative bacteria are generally less sensitive than Gram-positive due to the possession of the outer membrane containing porins, lipopolysaccharides, and lipoproteins resulting in a densely packed negative charge. This acts as a barrier to penetration, with only hydrophilic compounds with a molecular weight below 600-700 Daltons able to diffuse through the porin channels [91, 92]. It has been suggested in a recent study that Gram-negative bacteria may be more susceptible to killing induced by type I activation of photosensitisers, leading to the generation of hydroxyl radicals, whilst Gram-positive may be more susceptible to $^1\text{O}_2$ [93]. It is therefore possible that in cases where bacteria display lower susceptibility to photosensitisers, the choice of photosensitiser used may be governed by the pathway of generation of reactive oxygen species. A number of photosensitisers, including the phenothiazinium dye methylene blue, have been noted to act via both pathways, producing both radicals and $^1\text{O}_2$ [93].

A number of methods have been successfully used to overcome reduced susceptibility due to permeability barriers. One of the most straightforward is the imparting of a positive charge to the photosensitiser. This approach has been found to produce broad-spectrum photosensitisers, resulting in inactivation of G⁺ and G⁻ bacteria, in addition to viruses, fungi and parasites [79]. The majority of photosensitisers used in PACT are therefore cationic.

Two particular groups of photosensitiser have been heavily investigated for their antimicrobial actions – the porphyrins and phenothiaziniums. The general structure of porphyrins is shown in Figure 3.

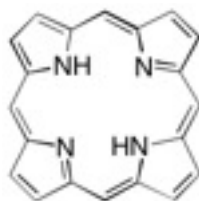
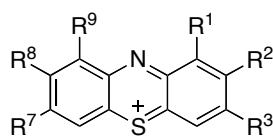


Figure 3. The structure of porphin, the parent compound on which porphyrins are based

Photosensitisers of the porphyrin class have received increased attention as, due to their presence in natural systems, they generally do not possess cytotoxicity in the dark. Furthermore, many possess long-lived triplet states, allowing for high quantum yields of $^1\text{O}_2$, and they can be easily modified with substituents, metal ions and ligands to allow optimisation of their properties [94]. Accordingly, a number of synthetic porphyrins have been designed and tested to achieve efficient microbicidal activity.

The general structure of phenothiazinium compounds is shown in Table 1.

Table 1. Structure of a number of commercially available phenothiazinium photosensitisers used in PACT



Compound	R ¹	R ²	R ³	R ⁴	R ⁷	R ⁸	R ⁹
Methylene blue	H	H	N(CH ₃) ₂	H	N(CH ₃) ₂	H	H
Toluidine blue O	H	H	NH ₂	H	N(CH ₃) ₂	H	H
New methylene blue	H	CH ₃	NHEt	H	NHEt	CH ₃	H
Dimethyl methylene blue	CH ₃	H	N(CH ₃) ₂	H	N(CH ₃) ₂	H	CH ₃

Phenothiaziniums have many favourable properties, conferring broad-spectrum antibacterial activity. They are cationic at physiological pH, allowing targeting of the negatively charged bacterial membrane, and their lipophilicity allows for partitioning into the membrane environment. These properties allow breach of the membrane as a barrier, and appear to enhance their efficacy against Gram-negative bacteria. It has also been suggested that toluidine blue O may induce structural changes in the lipopolysaccharide of the Gram-negative membrane, further reducing its barrier function [95]. Methylene blue and toluidine blue O have been suggested to form neutral quinonemine intermediates in the lower pH of the membrane, thus promoting their uptake through the membrane, possibly followed by regeneration of the cationic species intracellularly [71]. The planar, cationic structure of this class of photosensitisers allows intercalation with DNA nucleosides, followed by photo-oxidation, providing an important mechanism of phenothiazinium photoinduced cell

death. As with many photosensitisers, the primary sites of action of a given phenothiazinium can, however, differ between bacterial genera [22]. New methylene blue and new methylene blue N are effective against the Gram-negative bacterium *Yersina enterocolitica* which colonises red blood cell concentrates, conferring potential utility in blood disinfection [96], and as already mentioned, have demonstrated efficacy against antibiotic-resistant bacterial strains [73, 75].

3.3. Clinically used PACT

As with PDT, despite a wide research base and significant efforts in optimisation of photosensitiser design, only a small number have been used clinically for antimicrobial applications, but due to the success of published research, and increasing antibiotic resistance, it is anticipated that their clinical use will increase substantially over the coming years. Those used are of the porphyrin and phenothiazinium class, and neutral red, in addition to a conjugate between chlorin(e6) and polyethylenimine [79, 97]. The structure of neutral red is shown in Figure 4 .

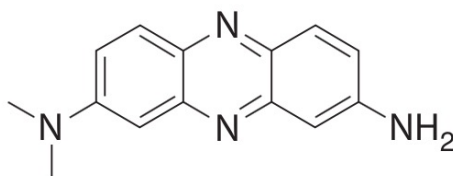


Figure 4. Structure of neutral red

Delivery of the photosensitiser to the target site also poses a challenge, and at present those used clinically are currently limited mainly to the topical treatment of localised

or dermatological infections (mainly ALA), or in dentistry (phenothiaziniums). As will be discussed, however, advances in the design of polymeric carriers for photosensitisers are anticipated to change this pattern in the future.

A summary of some infectious diseases that have been clinically treated with PACT is shown in Table 2.

Table 2. A summary of some infectious diseases that have been clinically treated using PACT

Infectious disease	Site of infection	Causative organism	Photosensitiser	References
Acne vulgaris	Skin and sebaceous glands	<i>P. acnes</i>	ALA Methylaminolevulinate Chlorophyll Indocyanine green	[98-103] [104-106] [107] [108]
Rosacea	Skin	Unknown	ALA Methylaminolevulinate	[109] [110]
Periodontitis	Dental pockets, gingival tissue	<i>Porphyromonas gingivalis</i> , <i>Fusobacterium nucleatum</i>	Phenothiazinium dyes including methylene blue	[111-115]
Peptic ulcer disease	Stomach	<i>Helicobacter pylori</i>	ALA Endogeneous porphyrins	[116] [117, 118]
Generic localised infections	Brain abscess	Bacteria	Haematoporphyrin	[79]
Onychomycosis	Nails	<i>Trichophyton</i> species	ALA	[88, 119, 120]
Cutaneous Leishmaniasis	Skin	Protozoa	ALA Methylene blue	[121-123] [88]
Herpes keratitis	Cornea	Herpes simplex virus	Proflavine	[124]
Genital herpes	Skin, mucous membranes	Herpes simplex virus	Methylene blue, neutral red, proflavine	[58]
Verruca vulgaris (common wart)	Skin	Human papilloma virus	ALA	[125-127]

As shown in Table 2, whilst applications have mainly been limited to topical treatments, PACT has allowed successful intervention in a large number of infectious diseases. One of particular note is the treatment of acne vulgaris. Acne vulgaris is not solely a microbiological issue, with many other contributing factors, however the presence and involvement of the bacterium *P. acnes* provides one mode of treatment. The haem biosynthetic pathway of ALA conversion to protoporphyrin IX is highly conserved across microorganisms [19], allowing a non-specific targeting of colonising microorganisms. *P. acnes* is known to accumulate high levels of porphyrins, rendering it particularly susceptible to ALA-mediated photoinactivation. For a thorough review of the use of photosensitisers in acne treatment, reference can be made to Wainwright *et al.* [128].

Another area being increasingly explored, and gaining rapid support of dental clinicians is the treatment of dental infections. Periodontitis is one of the most common bacterial diseases in humans, arising from the accumulation of plaque biofilms on the teeth and soft tissue of the mouth, and is frequently accompanied by inflammation of connective tissue and resorption of alveolar bone [79]. Phenothiazinium photosensitisers are injected into the affected area, usually the dental pocket, followed by a short period of illumination using a fibre optic tip. A number of companies have developed and marketed systems particularly for this use, and for treatment of infected root canals and tooth surfaces [129-131]. Considerable success has been demonstrated in the treatment of chronic [114, 115, 132-134], aggressive [67, 111, 113, 135], and HIV-associated [136] periodontitis in a number of patients, either as monotherapy or as an adjunct to conventional treatments, with results comparable to or superior to those of conventional treatments, and reduction of pain

and minimisation of the use of anaesthesia amongst the benefits. The reported improvements in clinical parameters such as pocket depths are mainly short-term, with further study required to fully ascertain long-term benefits.

A number of systems have been and are currently undergoing clinical trials for treatment of a variety of infections. Methylene blue systems are undergoing clinical trials for reduction of resistant endotracheal tube biofilms [137], and for decolonisation of nasal MRSA [138], and photodisinfection treatment of chronic sinusitis [139], both developed by Ondine Biomedical. HIV-associated oral candidiasis has also been successfully phototreated with methylene blue [140].

For a comprehensive description of clinical applications, readers are referred to reference [79].

3.3.1. Decontamination of blood

A number of disadvantages are associated with the use of conventional inactivation of pathogens in blood and blood products. UV light has been shown to damage plasma components, and may generate free radicals in plasma proteins, although the latter may be circumvented by the concomitant addition of antioxidants. Detergents cannot be used for blood disinfection due to potential damage to the erythrocyte cell membrane, and whilst physical methods, including filtration and washing, can remove extracellular contaminants, they are ineffective removal methods for intracellular pathogens [71]. Employing photodynamic inactivation of contaminating pathogens may afford a safer alternative.

Treatment of microorganisms within a human host poses a number of challenges, however it is possible to treat blood and blood products externally, prior to transfusion into a patient. Blood and blood products may be infected with bacteria, viruses including the human immunodeficiency virus (HIV), protozoa, or fungi and require disinfection prior to transfusion [71, 96]. The use of photosensitisers as disinfecting agents is recognised, and methylene blue has been widely used by a number of blood transfusion services in the decontamination of blood plasma, with particular efficacy against viruses [22]. As it absorbs at 656 nm [71], activation is with long wavelength light, which is not absorbed by haemoglobin or plasma, and thus can penetrate to activate methylene blue.

3.3.2. Novel areas and strategies in PACT

A number of photosensitisers and approaches to PACT have not yet been used clinically, but the findings of some recently published studies are of great interest and significance to the area of PACT, and are presented in the following sections.

3.3.2.1. Nebulised methylene blue for lung delivery

Delivery of the light source to the area to be treated can be problematic. A pilot study has been conducted, demonstrating nebulised delivery of methylene blue to a porcine CF lung and irradiation using a fibre optic light source, highlighting the ability to deliver a photosensitiser and light to the site of infection [141]. Antimicrobial susceptibility was not determined in this study, however previous studies have

demonstrated efficacy of PACT against lung pathogens, and the same group have assessed in a separate *in vitro* study the efficacy of methylene blue alone and in combination with antibiotics against *Burkholderia cepacia*, with positive results [142]. Due to the inherent resistance of *B. cepacia* to multiple antibiotics [143], this may provide a useful alternative. Clinical studies are required to verify the utility of such an approach.

3.3.2.2. *Immobilised photosensitisers*

3.3.2.2.1. *Immobilisation on polymers*

As reviewed, traditional PACT mainly requires uptake of the photosensitiser by the microorganism being challenged. Until recently, very few studies providing photoinactivation of microorganisms without the requirement for photosensitiser uptake had been published with application in pharmaceutical areas. Successful immobilisation of photosensitisers onto a support allows delivery to areas previously inaccessible by solutions. The contrast between the mechanism of action of conventional PACT and surface immobilised photosensitisers is shown in Figure 5.

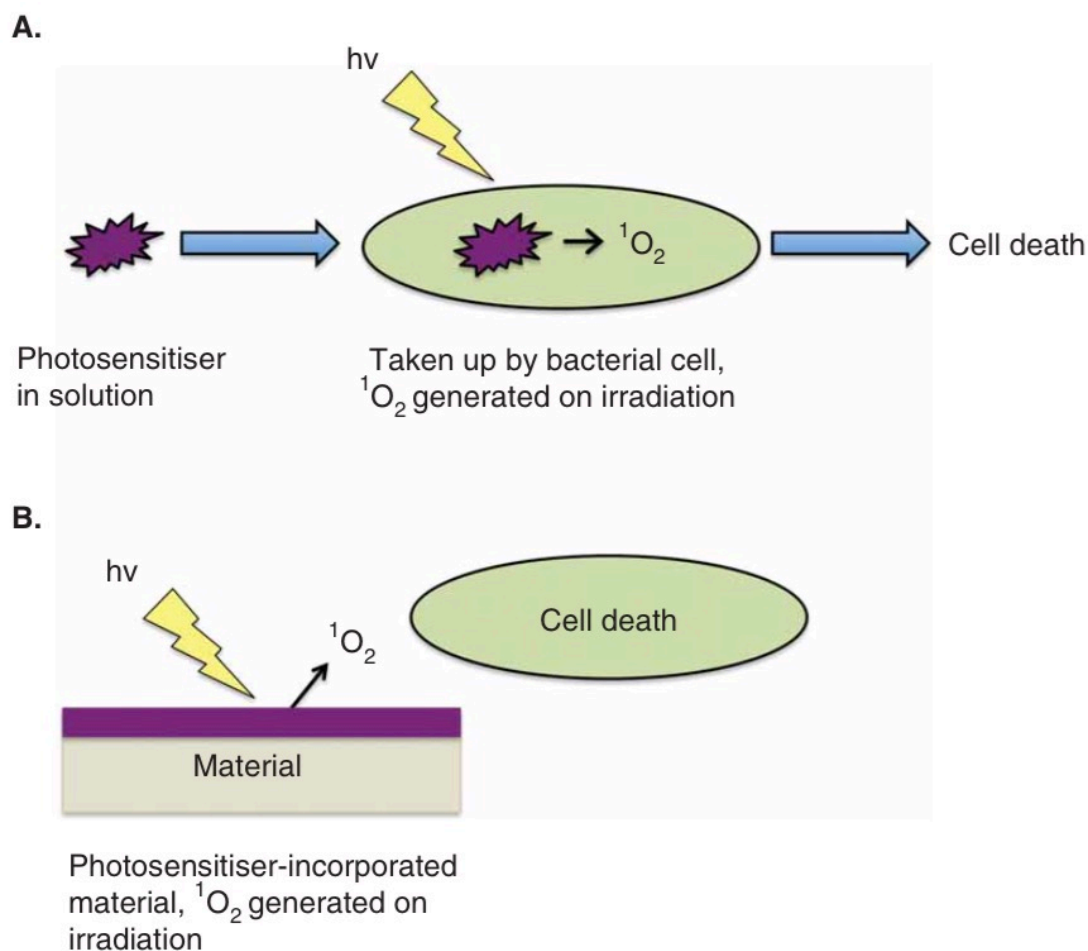


Figure 5. Comparison between the mechanism of conventional PACT (a) and surface-immobilised photosensitisers (b). Uptake of the photosensitiser is required by the bacterial cell in PACT, which then generates bactericidal reactive oxygen species such as $^1\text{O}_2$ on irradiation. With surface-immobilised photosensitisers, $^1\text{O}_2$ is generated from the surface of the material, exerting a cidal action on approaching bacterial cells when within the required distance. In both (a) and (b), photosensitiser is shown in purple

One of the earliest studies of immobilised photosensitisers was by Kautsky and de Bruijn who demonstrated that a solid impregnated with Rose Bengal generated what is now known to be $^1\text{O}_2$ (cited in [144]). Bonnett *et al.* in 1993 prepared polymer-porphyrin films by impregnating regenerated cellulose films with porphyrins [144],

by covalently binding porphyrin to the cellulose then casting, and by co-polymerising porphyrin with cellulose before casting, and subsequently demonstrated photobactericidal effects towards *S. aureus*, *Escherichia coli*, *Bacillus subtilis*, and *P. vulgaris* [145]. Since then, only a handful of studies have been conducted, but these provide important information regarding the potential for such an approach clinically.

A number of those published are related to work within the McCoy research group [20, 146, 147], whereby the photosensitiser is electrostatically localised at the surface of a polymer to prevent microbial adherence, with particular focus on ocular applications. Generated $^1\text{O}_2$ from the immobilised photosensitiser prevents bacterial adherence to the surface of the material, therefore preventing colonisation and biofilm formation. As the lifetime of $^1\text{O}_2$ is in the range of 10^{-5} – 10^{-6} seconds, the effective distance between the initial excitation event and cytotoxic damage is limited to a few micrometers, therefore preventing toxicity to normal tissue [20]. This is a sufficient distance to prevent bacterial adhesion, as reinforcement of adhesion to a surface is not thought to occur until a bacterium is within 1 nm of the surface [148]. High levels of surface localisation were achieved, coupled with promising antimicrobial activity against both Gram-positive and negative species.

Krouit *et al.* published two studies on the development of covalently bound porphyrin-cellulose films, using protoporphyrin IX [149] and monopyridyltritolylporphyrin [150] respectively to produce photobactericidal films. In both studies, $^1\text{O}_2$ -mediated inactivation of *S. aureus* and *E. coli* was reported, however as this was measured by the presence or absence of colonies on seeded nutrient agar plates after contact with the films under irradiation, the initial bacterial

challenge and log reduction are unknown. Also working with cellulose, but for applications as a surface coating is Wilson [151]. Toluidine blue O-incorporated cellulose acetate was challenged with Gram-positive and Gram-negative microorganisms, demonstrating a 4 and 5 log reduction of *S. aureus* and *E. coli* respectively following 24 hours irradiation with white light. This has the potential to be used as an operating surface coating or wall paint to reduce the spread of nosocomial infection. Further work by the same group involved incorporation of toluidine blue O or methylene blue into polyurethane and polysiloxane polymers by a swell-shrink method, in the presence and absence of gold nanoparticles, to achieve high photosensitiser loading [152, 153]. Promising microbial reductions of greater than 3 log were observed, with a further augmentation of approximately 0.5 log in the presence of gold nanoparticles. Although the photosensitiser was incorporated by physical means, leaching from the material was not noted.

Funes *et al.* investigated the substitution patterns of cationic porphyrin derivatives, and applied this knowledge to the electrochemical generation of polymeric films composed of porphyrin units [154]. The porphyrin, 5,10,15,20-tetra(4-N,N-diphenylaminophenyl)porphyrin, was used alone or complexed with palladium (II) chloride (Pd(II)) to enhance its photodynamic action, and is shown in Figure 6.

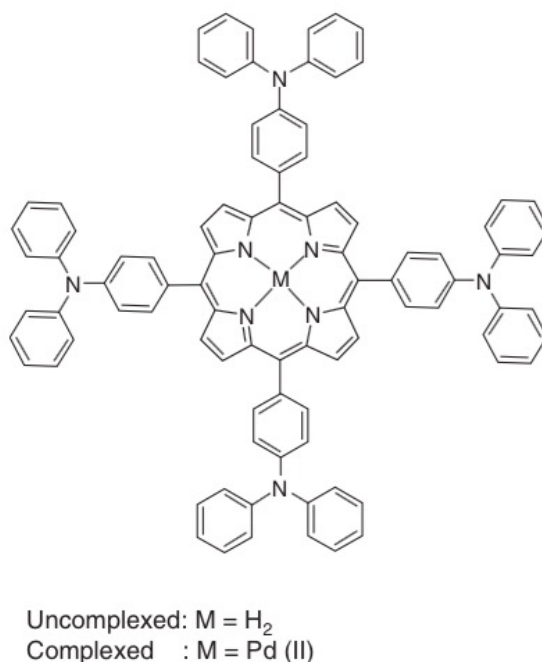


Figure 6. The porphyrin used by Funes *et al.* in the development of a porphyrin-containing antimicrobial film [154].

A 3 log reduction in *E. coli* viable count was noted following 30 minutes irradiation, with an approximately 2 log reduction in *C. albicans* viable count after the same period. There did not appear to be a significant difference between the complexed and uncomplexed porphyrin. The results obtained demonstrate the potential use of such films to inactivate microorganisms in liquid suspensions. It also highlights the need to continue to expand the range of photosensitisers being used.

Photosensitiser dyes bound to silica have also been studied, with modest success, primarily for applications in disinfection of blood and blood products [155], thus avoiding the challenge of removal of water-soluble photosensitisers from the medium following the photodisinfection procedure.

5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin has been incorporated into polysilsesquioxane polymer films and challenged with *Candida albicans*, achieving a 2.5 log reduction in viable count following 60 minutes of irradiation. This was substantially lower than that achieved elsewhere with cationic porphyrins in solution, however it does provide further demonstration of the anti-fungal properties of immobilised porphyrins. The proposed application of the films was decontamination of biological fluid or in the formation of antifungal surfaces in healthcare settings [156]. The log reductions in viable count are not sufficient for effective decontamination of surfaces or fluids, but it is possible that the use of a different photosensitiser, or a photosensitiser combination, may enable the design and fabrication of a film for use as a broad spectrum antimicrobial surface for application in healthcare facilities.

Other studies on photosensitiser immobilisation include grafting of cellulose fabric with porphyrin for use as a photobactericidal textile, although the reductions achieved in *S. aureus* and *E. coli* viable counts were modest [157]. A more recent study by Arenbergerova *et al.* [158] built on previous work by the same group [159] to develop porphyrin-doped electrospun nanofibre polyurethane textiles for use as wound coverings for leg ulcers. Light from a white fibre optic source was applied twice daily. The clinical study demonstrated a significant reduction in wound bacterial burden at day 28 and 42, and a decrease in wound size and wound-related pain in comparison to controls. This may provide an alternative to the topical application of antiseptics and antibiotics, to which resistance may develop.

A number of studies have been carried out employing immobilised photosensitisers for use in water decontamination [160-163], and for decontamination of food packaging and preparation surfaces [83, 164], however it is feasible that such systems could be transferred to biomedical and pharmaceutical applications.

3.3.2.2.2. Immobilisation on nano-scale polymers

Nanotechnology is a growing area, with innumerable potential applications. A growing number of studies involving photosensitisers demonstrates the breadth of potential. Porphyrin-cellulose nanocrystals, structure shown in Figure 7, following 30 minutes visible light illumination have demonstrated 5-6 log reductions in viable count of methicillin-resistant *S. aureus*, and multidrug-resistant *Acinobacter baumannii* [165].

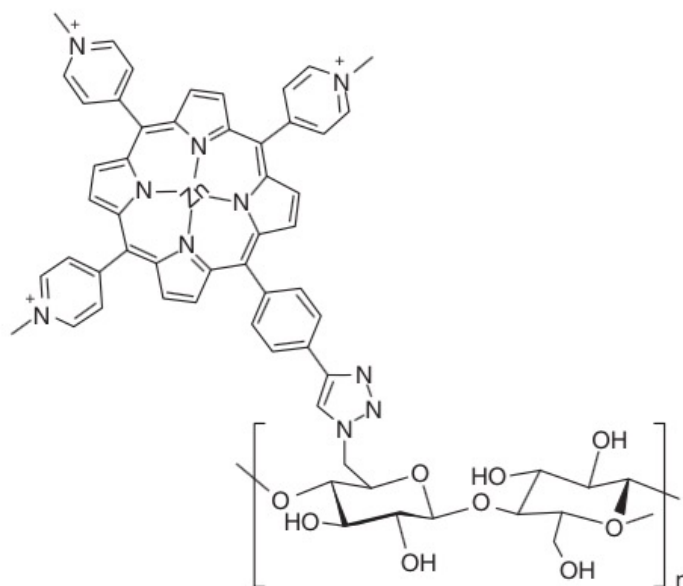


Figure 7. The structure of antibacterial porphyrin-cellulose nanocrystals developed by Carpenter *et al.* [165].

Only an approximately 2.5 log reduction of *P. aeruginosa* was achieved, which alongside observation of the previous results with *E. coli* [166] may suggest that this is a less effective strategy against Gram-negative bacteria, however the results are promising and display potential as an effective alternative to conventional antibacterial treatments.

Rose Bengal-functionalised chitosan nanoparticles have been studied for elimination of bacteria in the root canal, demonstrating significant efficacy against *Enterococcus faecalis* in the presence of tissue inhibitors following irradiation at 540 nm, owing to a combination of $^1\text{O}_2$ and the inherent anti-adherent properties of the positively charged material [167]. Further studies demonstrated anti-biofilm activity, with an additional benefit of dental collagen stabilisation [168], highlighting the potential clinical relevance of this system.

These studies firmly demonstrate the ability of photosensitisers to mediate photodynamic inactivation of microorganisms when incorporated into a number of materials, whether by covalent or non-covalent bonding, and highlight the potential for use as broad-spectrum antibacterial systems for prevention or treatment of infection. Whilst the studied nanoparticulate systems may provide a high surface area for $^1\text{O}_2$ generation, this may be difficult to reproduce for medical device polymers, for example. Therefore, the potential applications of immobilised polymers are dependent on the carrier onto which they are attached, or in which they are contained. Regardless of the carrier, it is anticipated that research in the area of immobilised photosensitisers will continue to grow and reach a clinical level, whereby infection

resistant materials can become an integral part of the wider infection control procedures in healthcare settings.

3.4. Photocatalytic disinfection

Whilst not employing photosensitisers for their action, the related area of photocatalysis must be mentioned for completeness. The most frequently-used photocatalyst is TiO_2 , an inexpensive nontoxic, chemically stable semiconductor catalyst with high photostability. Absorption of a photon of light leads to electron promotion from the valence to the conduction band, leaving a positively charged hole in the valence band. The photogenerated hole and electron can act as oxidation and reduction species respectively, leading to the generation of hydroxyl and hydroperoxyl radicals, and H_2O_2 . Three main polymorphs of TiO_2 exist: anatase, rutile and brookite. Anatase has been shown to be the most effective, and has a longer lifetime than the rutile form. The energy required for electron promotion, the band gap energy, allows UVA activation of anatase photocatalysis (below $\sim 385\text{ nm}$) [169]. Reactive oxygen species-mediated membrane and cell wall damage, involving lipid peroxidation and leakage of cellular components [170], appears to be the main mechanism of bactericidal action, and it has been shown to lead to complete cell mineralisation [171]. A number of bacterial, fungal, protozoal, algal, and viral strains have been shown to be killed by TiO_2 photocatalytic disinfection, an extensive list of which has been published by Foster *et al.* (2011) [169]. In order to shift the band onset of TiO_2 to allow activation in the visible light range, metal ions have been doped into the structure. Those studied include Ag and Cu, which have antimicrobial

effects that act in synergy with those of TiO₂, and a number of other transition metals, as reviewed by Liou and Chang [172].

A number of TiO₂ immobilised self-cleaning antimicrobial surfaces have been developed such as urinary catheters [174, 175] and orthodontic wires [176, 177], in addition to decontamination of drinking water [178, 179]. For a thorough review of antimicrobial applications of photocatalytic disinfection, readers are referred to Gamage and Zhang (2010) [180]. The photocatalytic antimicrobial effect of a TiO₂ nanocomposite has recently been shown to persist for up to two hours following removal of the UV light source [173]. This may open new avenues for research and application of TiO₂ materials for antimicrobial purposes where application of a constant light source is impractical.

4. Conclusions

Substantial advances have been made in the application of photosensitisers to the treatment of human disease and infection, and the ability to excite with light in the visible range provides a number of advantages. The current commercial use and clinical efficacy of a number of systems highlights the relevancy of research on these compounds and associated delivery systems. Whilst the majority of success for both photodynamic therapy, and photodynamic antimicrobial chemotherapy has been seen with ALA, and photosensitisers of the porphyrin and phenothiazinium class, current research with other compounds suggests that future clinical applications may be afforded from a wider range of sensitisers, both free and immobilised onto bulk and

nano-scale polymeric systems, thus expanding the potential applications for photosensitising compounds, particularly in the area of antimicrobials.

5. Expert Opinion

The use of light with photosensitisers provides a means by which treatment can be highly controlled in terms of time, space, and duration. This allows tailoring of cancer treatments and antimicrobial strategies. In terms of antimicrobial action, the multiplicity of targets in the microbial cell, and demonstrated lack of resistance despite attempts to induce it, confer a great benefit over conventional antimicrobial methods and disinfectants. Given the findings of the recent WHO report [68], highlighting the global problem of antibiotic resistance, the potential of photosensitiser-mediated systems is therefore of significance. Particularly, WHO highlighted *E. coli* antibiotic resistance, associated with urinary tract infections, high levels of methicillin resistance in *S. aureus*, and resistance to the last resort (carbapenem) treatment for *Klebsiella pneumonia*, often associated with hospital-acquired pneumonia. As prevention is often a preferable strategy to cure, photosensitising systems such as those described show significant promise for infection prevention. While the existing clinical strategies employing photosensitisers have already shown great efficacy, and are of great utility in the treatment of a number of conditions, they are somewhat limited due to the currently narrow range of photosensitisers used, and the use of photosensitisers mostly in solution. A current goal in the field is to expand the range of indications for PDT, and this may be enabled by the development of new photosensitiser constructs capable of enhanced tissue targeting, or which can be activated by longer wavelengths of light in two-

photon processes, allowing deeper tissue penetration and fewer side effects, particularly in damage to healthy tissue, and also antimicrobial treatment of tissue areas not normally directly accessible to light.

It is anticipated that, with the synthesis of newer generation photosensitisers, and design of novel delivery strategies and carriers, the clinical applications of photosensitisers will further expand to the prevention and treatment of infection, and will be of significance in at least partially addressing the wider global issue of bacterial resistance. Their use holds the potential to reduce or replace the use of conventional surface decontamination methods, and of antibiotics for some infections. Alongside this, however, as with any antimicrobial strategy, is the necessity to assess the continuing evasion of bacterial resistance to provide continued assurance of the superiority of these compounds over conventional treatments.

Movement towards surface immobilisation has opened and will continue to open avenues for treatment of hospital-associated infections, related both to those carried on hospital surfaces and on medical devices. By providing a means by which bactericidal action can be exerted, without the requirement for uptake of the photosensitiser, the issue of surface contamination and biofouling can be addressed. The experimental microbial reductions seen with immobilised photosensitisers are significant, and hold promise for the development of clinically useful devices and surfaces. It is likely that with continued research to achieve optimal loading concentrations and light doses, and therefore singlet oxygen generating efficiency, a number of such photosensitiser-incorporated materials and medical devices will be developed for clinical testing over the coming years. These will have the potential to

revolutionise device use, with only light being required for persistent decontamination, and enabling prevention of surface colonisation before infection can result. Bringing together the fields of polymer and material science with photosensitiser design and development will allow fabrication of novel and diverse materials, with efficacy resulting both from optimal material properties and optimal photosensitising efficiency. Application of light of multiple wavelengths may allow stimulation of different photosensitiser classes on one device, or indeed allow the combination of photocatalysis with photosensitiser-mediated antimicrobial therapies. The future of light-activated photosensitiser research is therefore undoubtedly bright, and is likely to produce clinically beneficial applications in this growing field.

6. Declaration of Interest

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